Early Diagnosis of Primary Human Herpesvirus 6 Infection in Childhood: Serology, Polymerase Chain Reaction, and Virus Load

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Qualitative and quantitative polymerase chain reaction (PCR) for human herpesvirus 6 (HHV-6) DNA in whole blood and plasma was correlated with serology and clinical assessment in 143 children hospitalized for undifferentiated febrile illness to evaluate options for diagnosis of primary HHV-6 infection on the acute blood specimen. PCR and serology for HHV-7 were done in parallel to define serologic cross-reactions. Using HHV-6 seroconversion as the reference standard, detection of HHV-6 DNA in whole blood in the absence of antibody in the plasma was the most reliable evidence of primary HHV-6 infection. Detection of HHV-6 DNA in plasma and a high virus load in whole blood (>3.3 $\log_{10}$ copies/5 μL) had a sensitivity of 90% and 100%, respectively, in diagnosing primary HHV-6 infection. However, both were occasionally found in patients with other infections, possibly associated with HHV-6 reactivation. Maternal antibody may confound interpretation of serology in patients under 3 months of age.

Human herpesvirus 6 (HHV-6) is a common infection in childhood. Primary infection usually occurs during infancy [1], and 76%–92% of children are seropositive by the age of 1 year [2]. Primary infection is associated with the syndrome roseola infantum or exanthem subitum [3], an acute febrile illness followed by a maculopapular rash appearing with defervescence. Clinically, this diagnosis becomes apparent only in retrospect with the appearance of the rash. Furthermore, the typical presentation of roseola is seen only in a proportion of patients, and the full clinical spectrum of primary HHV-6 infection is still being elucidated. In one study, it accounted for 20% of visits to pediatric emergency services for febrile illness in children 6–12 months of age [4]. The majority of these patients did not present with classical roseola, and at presentation to pediatric outpatient or inpatient services, a wide range of differential diagnoses were considered, including otitis media, sepsis, and meningoencephalitis. Furthermore, many children clinically diagnosed to have measles or other exanthems in fact have HHV-6 infections [5].

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Informed and written consents were obtained from the patients' parents or guardians prior to enrollment in the study, and the protocol was approved by the Ethics Committee of the University of Hong Kong.

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The only options for laboratory diagnosis available at present are the demonstration of rising antibody titers (for which paired specimens of blood are required) and culture of the virus from peripheral blood mononuclear cells [3]. In both instances, the diagnosis is retrospective. Furthermore, viral culture for HHV-6 requires cocultivation with cord blood mononuclear cells and is not practicable in a routine diagnostic laboratory setting. Antibody avidity has been investigated for the diagnosis of primary HHV-6 infection, but since antibody is not detectable in specimens collected within the first 5 days after onset of illness, it is only applicable in convalescent sera or those collected later in the illness [6]. Similarly, IgM antibody detection has thus far not proved to be sensitive and specific enough for routine diagnosis when applied to the acute blood specimen. More than 25% of serum specimens collected at initial presentation may be negative for HHV-6 IgM antibody [7]. Typically, the IgM response develops 5–7 days after onset of the disease.

Detection of viral DNA in plasma by polymerase chain reaction (PCR) has been reported previously as a diagnostic option [8, 9]. However, in the study by Huang et al. [8], only a minority of patients with acute HHV-6 infection had viral DNA detected in the acute plasma specimen, although the convalescent specimen was always positive. The study by Secchiero et al. [9] investigated few ($n = 7$) pediatric patients, and details on timing of specimens in relation to onset of disease was not reported. Thus, the sensitivity, specificity, and predictive value of plasma DNA detection in the diagnosis of primary HHV-6 infections remain undefined at present. A recent study [10] has investigated qualitative and quantitative PCR in peripheral blood leukocytes (PBL) and saliva for the diagnosis of primary HHV-6 infection. However, they rely on the yet-unproved hypothesis that detection of viral HHV-6 DNA in PBL in the absence of HHV-6 DNA in saliva indicates primary HHV-6

infection. None of the 3 patients had the diagnosis of primary HHV-6 infection confirmed by seroconversion or viral culture.

The aim of the present study was to define laboratory parameters that can be used to diagnose primary HHV-6 infection by testing the acute specimen (within 5 days of disease onset) collected at the time of initial presentation.

The hypotheses being tested were that in the acute blood specimen, the following were predictive of primary HHV-6 infection: detection of HHV-6 DNA in plasma, a profile of detectable DNA in whole blood in the absence of IgG antibody in the plasma, and a higher viral DNA load in the whole blood specimen. Further, we hypothesized that the HHV-6 DNA/IgG profile of the acute blood specimen would correlate with infection status in the following way: HHV-6 DNA detected in whole blood in the absence of HHV-6 IgG in plasma correlates with primary HHV-6 infection, positive HHV-6 IgG (irrespective of viral DNA result) is indicative of past HHV-6 infection, and a negative result for both HHV-6 DNA and IgG indicates the absence of infection (past or present).

Methods

Patients. Children aged ≤3 years admitted (to the general pediatric ward of a teaching hospital) with fever (>38.0°C) of <5 days' duration were eligible for recruitment to the study. Patients with an obvious bacterial etiology for fever (e.g., lobar pneumonia, urinary tract infection) at admission were excluded. After discharge from the hospital, patients were requested to return 2–4 weeks later for clinical and serologic follow-up. Review of patient charts was done by one of us (S.S.C.) without prior knowledge of the laboratory results.

Specimens. During routine clinical workup of the patients, an extra 0.5 mL of blood was drawn into EDTA for the purposes of HHV-6 and HHV-7 serology and PCR analysis. The specimens were centrifuged, and 200 μL of plasma was withdrawn and stored at −70°C. The volume of the remaining blood specimen was reconstituted by adding back 200 μL of PBS, and the reconstituted whole blood was stored frozen at −70°C.

DNA was extracted separately from 200 μL of the thawed plasma and the whole blood specimens with the Qiagen blood kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. When extracting viral DNA from plasma, 0.04 μg of carrier DNA was added to the 200-μL plasma sample before application to the QIAamp column. The DNA was eluted into 200 μL of autoclaved deionized water and stored at −20°C until tested by PCR.

Saliva specimens were collected from some patients at the time of hospitalization (Salivettes; Sarstedt, Numbrecht, Germany). The sponge was placed in the mouth for 45 s before being replaced in the container. Saliva was extracted by centrifugation and stored at −70°C. The saliva was boiled for 10 min, and the centrifuged supernatant was used for PCR.

PCR for HHV-6 and HHV-7 DNA. The primers used for HHV-6 [11] and HHV-7 [12] and the nested PCR conditions have been described previously [13]. DNA extract (5 μL) was amplified by a nested PCR in a 50-μL reaction mixture containing Taq polymerase (Boehringer Mannheim, Mannheim, Germany) and 1.5 m*M* MgCl. Agarose gel electrophoresis was used to detect the amplicons of the HHV-6 nested PCR. The identity of the HHV-7 amplicons were confirmed by oligonucleotide hybridization as described previously [13], with the modification that a digoxigenin-labeled probe was used together with a chemiluminescent detection system (Boehringer Mannheim).

Precautions to exclude cross-contamination during specimen extraction and PCR amplification [14] were strictly adhered to. The PCR for HHV-6 and HHV-7 did not cross-amplify the genomes of each other or that of HHV-1 through HHV-5 [13].

Quantitative PCR for HHV-6 DNA. Specimens positive for HHV-6 DNA in the screening PCR assay were quantitated by an end-point dilution PCR. Serial 0.5-log dilutions of the DNA extracts were tested in a single-round hot-start PCR reaction with the outer PCR primers and Amplitaq Gold (Applied Biosystems, Foster City, CA). Each PCR run included a dilution series of the linearized plasmid pH6 [11] equivalent to 3.8, 3.3, 2.8, and 2.3 $\log_{10}$ genome copies. The band intensities of the amplified diluted specimens were compared with those of the standard dilution curve to estimate the number of genome copies in each sample. Variation between PCR runs was within 0.5 $\log_{10}$ genome copies. To further control for variability between PCR runs and make comparisons between patient and specimen groups more reliable, DNA extracts of acute and convalescent whole blood and plasma from a patient with HHV-6 seroconversion were titrated in parallel with DNA from whole blood of a matched patient with past infection.

The pH6 standard we used was compared with an independent plasmid pH6 preparation of known genome copy number (from W. Pumeechockchai, University of Newcastle upon Tyne, UK) and a quantified preparation of viral DNA extracted from purified HHV-6 nucleocapsids (provided by P. Pellet, Centers for Disease Control and Prevention, Atlanta).

Serology for HHV-6 and HHV-7. HHV-6 (AJ strain)–infected HSB-2 cells and HHV-7 (DC strain)–infected Sup-T1 cells were used in indirect immunofluorescence assays to detect HHV-6 and HHV-7 antibodies, respectively [13]. Patients' plasma specimens were screened at a dilution of 1/50 by an anti-human IgG conjugate (Inova, San Diego). In patients with paired specimens, serial 2-fold dilutions of plasma specimens were titrated in parallel, starting at a dilution of 1/25. All immunofluorescence results were read by the same investigator (M.P.).

Other laboratory investigations. The patients were investigated for other microbiologic causes of febrile illness as clinically indicated. These included examining nasopharyngeal aspirates for viral antigens by immunofluorescence and other appropriate specimens for viral and bacteriologic culture.

PCR analysis and serology were carried out blinded to the results of each other, to other routine laboratory investigations, and to the clinical assessment of the patient.

Results

One hundred forty-three patients were recruited over an 8-month period between November 1996 through June 1997. Paired blood specimens were available from 49 patients. The remaining 94 patients failed to attend follow-up for convalescent specimen collection and were analyzed separately. The pa-

tients with paired and single blood specimens were similar in demographic and clinical parameters: mean age, 12.2 versus 13.6 months ($P = .3$); male-to-female ratio, 1.5:1 versus 1.4:1 ($P = .7$); duration of fever at admission, 2.0 versus 1.8 days ($P = .27$); and mean temperature at presentation, 39.4°C versus 39.3°C ($P = .3$).

To minimize the potential confounding effect of maternal antibody on serologic responses, patients with paired blood specimens who were >3 months old at presentation ($n = 42$) were analyzed initially. Twenty patients had ≥4-fold increases in antibody titer to HHV-6, 18 patients had static titers to HHV-6, and 4 patients remained seronegative in both acute and convalescent specimens.

Using HHV-6 seroconversion as the reference standard for diagnosing primary or past HHV-6 infection, we evaluated the detection of HHV-6 DNA in whole blood, the detection of HHV-6 DNA in plasma, and the HHV-6 DNA/IgG profile as methods for providing a reliable diagnosis on the acute specimen at initial clinical presentation (table 1). On the basis of these results, the sensitivity, specificity, positive predictive value, and negative predictive value of detecting viral DNA in acute whole blood for diagnosing primary HHV-6 infection were 100%, 41%, 61%, and 100%, whereas those for DNA in the acute plasma were 90%, 100%, 100%, and 92%, respectively. One of the 20 patients with primary infection and another with past infection had detectable viral DNA in the convalescent plasma specimen.

The profile of positive HHV-6 DNA in the absence of IgG in an acute whole blood specimen had sensitivity, specificity, and predictive values of 100% for diagnosing primary HHV-6 infection.

Quantitation of viral DNA in whole blood and plasma. The use of hot-start PCR (Amplitaq Gold) in the quantitative PCR significantly reduced nonspecific amplification bands often seen when using conventional Taq polymerase enzymes and resulted in improved sensitivity and specificity of the single-round PCR reaction, eliminating the need to use nested PCR to confirm specificity of the PCR reaction. Figure 1 shows the HHV-6 DNA load (genome copies/5 μL) in acute and convalescent whole blood of patients with HHV-6 seroconversion and past infection, respectively. In patients with HHV-6 seroconversion, viral DNA load in the acute whole blood specimen was at least 0.5 $\log_{10}$ greater than that in the convalescent specimen. In contrast, those patients with serologic evidence of past infection had a ≤0.5 $\log_{10}$ difference in virus load between acute and convalescent specimens with 1 exception (the latter being associated with a 1 $\log_{10}$ increase in the convalescent specimen). All patients with seroconversion to HHV-6 had >3.3 $\log_{10}$ copies of HHV-6 DNA/5 μL of acute whole blood, in contrast to those with past infection (figure 1). This level of virus load differentiated recent from past infection with sensitivity, specificity, and predictive values of 100% (table 1).

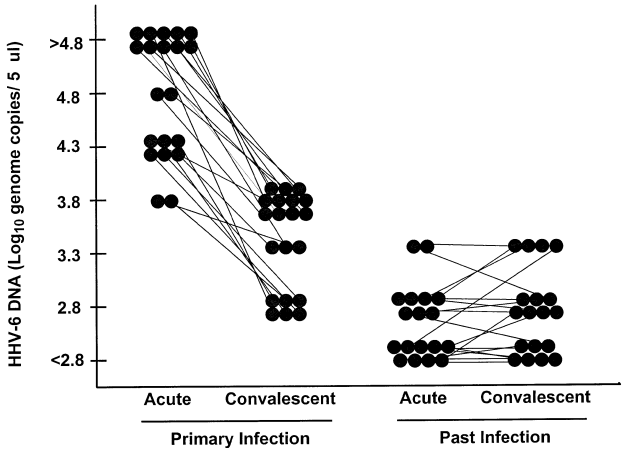

Figure 1. HHV-6 DNA load (genome copies/5 μL) in whole blood specimens: comparison of acute and convalescent specimens in primary and past infections.

Five patients with primary HHV-6 infection had a third blood specimen collected between 22 and 29 weeks after onset of primary infection, at which time viral DNA load in all but 1 patient had fallen to ≤2.8 $\log_{10}$ copies/5 μL of whole blood.

Viral DNA levels in the acute whole blood specimen were compared with those in the corresponding plasma specimen (figure 2). Viral DNA levels in whole blood were usually higher than those in plasma.

Patients <3 months of age. The laboratory findings of the 7 patients <3 months of age whose paired blood samples were excluded from initial analysis were as follows. Two patients were seronegative in both acute and convalescent plasma specimens, were PCR-negative for HHV-6 in all specimens, and were therefore presumed not to have HHV-6 infection. In fact, 1 of them had a confirmed alternative diagnosis of *Escherichia coli* urinary tract infection. The remaining 5 patients all had

Table 1. HHV-6 DNA results on 42 patients >3 months old with paired sera.

Serostatus to HHV-6	HHV-6 DNA–positive		HHV-6 DNA–positive/IgG-negative in whole blood (acute specimen)	>3.3 $\log_{10}$ copies of HHV-6 DNA/5 μL of whole blood[a]	Seroconversion to HHV-7
	In whole blood	In plasma			
Seroconversion ($n = 20$)	20/20 (100)	18/20 (90)	20/20 (100)	20/20 (100)	8/20 (40)
Static titers ($n = 18$)	13/18 (72)	0/18	0/18	0/18	0/18
No antibody ($n = 4$)	0/4	0/4	0/4	0/4	0/4

NOTE. Data are no./total (%).

[a] Cutoff value for viral DNA load based on data in figure 1.

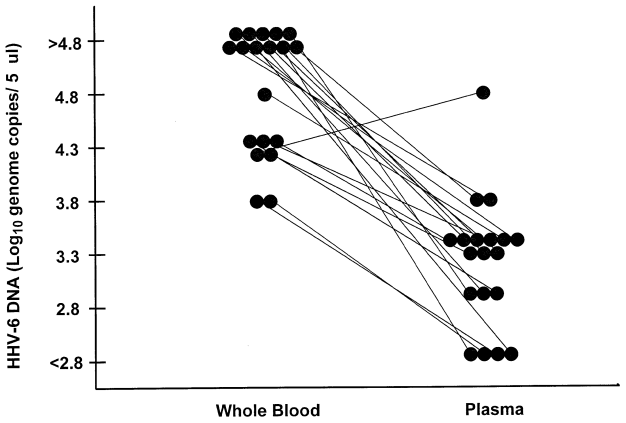

Figure 2. Comparison of HHV-6 DNA load (genome copies/5 μL) in acute whole blood and plasma specimens.

HHV-6 DNA in the acute whole blood DNA extract. Two of these patients seroconverted to HHV-6, had the HHV-6 DNA/IgG profile suggestive of primary HHV-6 infection, had high viral DNA loads in whole blood, and were clinically diagnosed to have roseola. However, unlike older children whose convalescent antibody titers often exceed 1/400, 1 of these young babies developed only modest antibody titers to the virus (1/50 at 2 weeks and 1/100 at 27 weeks after infection).

Two other patients remained seronegative in both acute and convalescent specimens, and 1 other had static antibody titers, although all 3 had high viral DNA load in the whole blood ($\geq$4.3 log genome copies/5 μL), had viral DNA in the plasma, and had a clinical diagnosis of roseola. The overall interpretation of these findings indicates that these 3 patients had primary HHV-6 infection, even though there was no detectable serologic response.

Serology to HHV-7. Of the 42 patients >3 months old for whom paired serum specimens were available, 8 had seroconversion to HHV-7 and a further 6 had a marginal serologic response (i.e., <1/25 to 1/25). Only 3 of these patients had HHV-7 DNA detected in the acute whole blood specimen, 1 of them also having HHV-7 DNA in the plasma. None of these 3 patients had HHV-7 IgG in the acute plasma specimen, and their clinical presentation was that of roseola in 1 and a febrile illness with diarrhea in the other 2.

All 14 patients had concurrent seroconversion to HHV-6, had detectable HHV-6 DNA in whole blood in the absence of IgG, and had high HHV-6 DNA load (>3.3 $\log_{10}$/5 μL of whole blood). Twelve had HHV-6 DNA in the plasma. None of the patients negative for antibody or with static titers to HHV-6 had a rise in antibody titers to HHV-7.

Patients from whom only a single (acute) blood specimen was available. Eighty-nine of the 94 patients from whom paired specimens were unavailable were >3 months of age. Of them, 14 had detectable HHV-6 DNA in the absence of IgG in the acute specimen. Seven of these children had a clinical diagnosis of roseola and a further 6 had a clinical diagnosis of a viral syndrome without a confirmed alternative microbiologic diagnosis (table 2). Only one 7-month-old child had a confirmed alternative diagnosis (parainfluenza croup). This patient also had HHV-6 DNA detectable in the plasma and a high viral DNA load in the blood. A follow-up blood specimen obtained 20 weeks later showed that he seroconverted to HHV-6 during this period.

In contrast, of 75 patients >3 months of age who had HHV-6 IgG present in the acute plasma (HHV-6 DNA/IgG profile suggestive of past infection) or were HHV-6 DNA– and IgG-negative (HHV-6 DNA/IgG profile of no infection), none had roseola, while 55 (73%) had a confirmed alternative microbiologic diagnosis.

Seventeen of the 89 patients had HHV-6 DNA detected in their plasma (table 2). Seven of these patients had clinical roseola, and all of them had detectable HHV-6 DNA in the absence of IgG (the profile hypothesized to be associated with primary HHV-6 infection). Seven other patients had HHV-6 IgG in the acute blood specimen (the profile hypothesized to indicate past infection), none of whom had roseola, but 5 had alternative microbiologic diagnoses (respiratory syncytial virus, 1; influenza A, 2; pneumococcal infection, 1; parainfluenza, 1). Three of the 5 patients with alternative diagnoses had HHV-6 DNA loads >3.3 $\log_{10}$/5 μL of whole blood.

Saliva specimens. Twenty-four of the 33 saliva specimens collected at initial presentation provided adequate specimen volume for study. All 11 specimens collected from patients whose whole blood was HHV-6 DNA–positive in the absence of IgG had no detectable HHV-6 DNA in saliva, whereas 7 of 8 specimens from patients who were seropositive at initial presentation had detectable HHV-6 DNA in their saliva. Of 5 patients negative for both HHV-6 DNA and IgG in blood, none had viral DNA in saliva.

Demographic data of the overall study population. When the HHV-6 DNA/IgG profile of the acute blood specimen was applied to the whole study population of 143 children, primary HHV-6 infection was diagnosed in 39 (27%) of the patients overall (under the age of 3 years) and 33 (50%) of the 66 patients under the age of 1 year. The demographic data on the study population with and without primary HHV-6 infection, respectively, were as follows: male-to-female ratio, 1.3:1 versus 1.3:1; median age, 7.4 versus 14.7 months ($P < .001$). Twenty-eight (72%) of the 39 children with laboratory evidence of primary HHV-6 infection had clinical roseola.

Of the 143 children, an alternative diagnosis was documented in 66, only 1 of whom had the HHV-6 DNA/IgG profile of primary infection. The alternative microbiologic diagnoses were influenza A and B, adenovirus, respiratory syncytial virus, parainfluenza viruses, bacteremia, *Salmonella*, rotavirus, *Campylobacter jejuni, Branhamella* pneumonia, urinary tract infection, and herpangina.

Table 2. Comparison of HHV-6 DNA/IgG profile and plasma DNA results in patients >3 months old (n = 89) with single blood specimens.

HHV-6 DNA/IgG profile in acute specimen	Plasma DNA in acute specimen	Roseola	Undiagnosed viral fever	Alternative microbiologic diagnosis
DNA-positive/IgG-negative (primary HHV-6 infection)	Positive	7	2	1[a]
DNA-positive/IgG-negative (primary HHV-6 infection)	Negative	0	4	0
IgG-positive (past HHV-6 infection)	Positive	0	2	5
IgG-positive (past HHV-6 infection)	Negative	0	13	33
DNA- and IgG-negative (no past HHV-6 infection)	Negative	0	6	16

NOTE. Parentheses indicate hypothesized interpretation of HHV-6 DNA/IgG profile.

[a] Croup associated with parainfluenza type 3. Subsequent convalescent serum showed seroconversion to HHV-6.

Discussion

To validate the diagnostic options under investigation, our initial analysis focused on patients >3 months of age with paired blood specimens (n = 42), and we used seroconversion as the reference standard to define primary HHV-6 infection. Serology and PCR for HHV-7 were also done in parallel, because this antigenically related virus may confound interpretation of HHV-6 serology.

Detection of viral DNA in acute whole blood specimens had a low positive predictive value (57%) for primary HHV-6 infection, because viral DNA persists in PBL for many months or years after primary infection. However, a positive viral DNA result in whole blood combined with the lack of IgG antibody in the corresponding plasma specimen proved to be a reliable indicator of primary HHV-6 infection, with excellent sensitivity, specificity, and positive and negative predictive values. The value of the HHV-6 DNA/IgG profile is further supported by the data from patients (>3 months of age) from whom only acute blood was available (table 2). While 72% of patients with a DNA/IgG profile of past HHV-6 infection had microbiologically confirmed alternative diagnoses, only 1 of 14 patients with a profile of primary infection had an alternative diagnosis. The latter patient had seroconverted to HHV-6 when recalled for convalescent blood sampling 20 weeks later, and he probably had concurrent infection with both HHV-6 and parainfluenza virus.

In a study in which sequential specimens of PBL, plasma, and saliva were studied in 3 patients with serologically proven primary HHV-6 infection, Suga et al. [15] found that viral DNA appears in saliva only during convalescence. In a subsequent study, others [10] have suggested that detection of HHV-6 DNA in PBL in the absence of viral DNA in the saliva may be used to diagnose primary HHV-6 infection. The latter study had no independent confirmation (e.g., seroconversion or viral culture) of primary HHV-6 infection, and conclusions were based on only 3 patients. Our study supports the contention that the absence of viral DNA in the saliva in the presence of HHV-6 DNA in the peripheral blood indicates primary HHV-6 infection. It must be noted, however, that more data need to be collected in this regard and also that saliva is a less readily standardized clinical specimen. These results also suggest that primary HHV-6 infection is initially a systemic infection, with the virus localizing to the salivary glands during convalescence.

The quantitative estimation of viral DNA in whole blood and plasma specimens from patients with HHV-6 seroconversion or past infection is shown in figures 1 and 2. Precautions to minimize bias from interexperimental variation on the comparison of virus load between acute versus convalescent specimens and primary versus past infections were taken. Viral DNA load in whole blood of patients with HHV-6 seroconversion was clearly higher than that of patients with past infection. A previous preliminary report [10] based on 3 patients had similar results. To exclude age-related bias, we quantified virus load in 6 other patients (mean age, 8 months) from whom only single specimens were available, who were viral DNA–positive in the screening PCR assay, and who were HHV-6 IgG–positive. All 6 had viral DNA loads of $\leq$3.3 $\log_{10}$ (data not shown). We conclude that virus load may be used for diagnosing primary HHV-6 infection in the acute blood specimen.

There are only two other reports on HHV-6 load in primary HHV-6 infection [9, 10]. Secchiero et al. [9] found virus loads ranging from 6×10^2 to 6×10^5/mL (0.48–3.5 $\log_{10}$/5 μL) in serum in 6 patients with exanthem subitum. Eighteen of our 19 patients with primary HHV-6 infection had comparable virus loads (<2.8–3.8 $\log_{10}$/5 μL). Clark et al. [10] report virus loads of 4.3–5.5 $\log_{10}/10^6$ PBL isolated from peripheral blood of 3 patients with primary HHV-6 infection. Our virus load in primary HHV-6 infection was determined on DNA extracted directly from whole blood rather than on PBL isolated from whole blood. The mean total leukocyte count in febrile children with primary HHV-6 was 7.4×10^9/L, in contrast to 13×10^9/L in children with past HHV-6 infection (Chiu SS, Peiris M, unpublished data). If our results are converted to virus load/10^6 PBL (based on each patient's total leukocyte count), they range between 4.9 and >6.5 $\log_{10}$, that is, ~10-fold higher than that reported by Clark et al. [10]. Similarly, their data for children with past infection show a lower virus load (2.5–3.9 $\log_{10}$) than ours (<3.8–4.7 $\log_{10}$). The separation of PBL before extraction of the viral DNA (rather than direct extraction from whole blood) may contribute to some of this difference in virus loads between our results and those previously reported [10]. We have preliminary evidence that separation and washing of

PBL before DNA extraction results in lower virus loads (Peiris M, Cheung CY, unpublished data).

Other published data on HHV-6 load pertain to healthy adults [16, 17] or human immunodeficiency virus–infected patients [18] and are not comparable with those for our patient group. When we examined blood from healthy adults, HHV-6 loads were $<2.8 \log_{10}/5\ \mu L$ (i.e., $<1.8 \times 10^4/10^6$ PBL) and clearly lower than those found in infants with past infection (Peiris M, Cheung CY, unpublished data). Previously published HHV-6 loads in healthy volunteers have ranged between 3×10^1 and $4 \times 10^3/10^6$ PBL [17], but HHV-6 load was as high as $1.2 \times 10^6/\mu g$ of DNA (i.e., $\sim 10 \times 10^6/10^6$ PBL) in 1 person [16]. When virus load data from different studies are compared, the methods of extracting the DNA, the methods of isolating the PBL (some leukocyte separation methods isolate only mononuclear cells while others also isolate granulocytes), and the patient populations must be considered.

Detection of HHV-6 DNA in the plasma has been proposed as a means of diagnosing primary infection in the early stage of the illness [8, 9], although the data supporting this assertion are sketchy. Among the 42 patients >3 months of age from whom paired blood was available, the sensitivity of detecting HHV-6 DNA in the plasma for diagnosing primary HHV-6 infection was 90%. The lower sensitivity of plasma DNA is explained by the lower virus load found in plasma compared with whole blood (figure 2). Only 1 convalescent plasma specimen (36 days after infection) remained HHV-6 DNA–positive, suggesting that HHV-6 DNA in plasma does not persist, in contrast to that in leukocytes.

While none of the 18 patients with past infection had detectable HHV-6 DNA in the acute plasma specimen, 1 of their convalescent plasma specimens was positive for HHV-6 DNA. This 2-year-old patient had an alternative diagnosis of influenza A. Furthermore, 7 other patients without paired specimens had detectable plasma DNA associated with an HHV-6 DNA/IgG profile suggestive of past infection; 5 of them had alternative microbiologic diagnoses (table 2). Four of these children had viral DNA loads usually associated with primary infection (i.e., $\geq 3.8 \log_{10}$). It is possible that reactivation of HHV-6 in response to another infection may lead to detectable HHV-6 DNA in the plasma as well as a high viral DNA load in whole blood. Given these results, the true positive predictive value of a positive HHV-6 DNA result in plasma for primary HHV-6 infection must be <100%. Presumptive evidence of other viruses stimulating HHV-6 reactivation in immunocompetent children has been previously reported [4]. The fact that HHV-6 DNA in the plasma may be associated with reactivation of HHV-6 in the immunocompromised patient is already recognized [13]. Definitive laboratory markers of viral reactivation, however, are still awaited.

Three of 5 patients under 3 months of age with clinical and virologic evidence of primary HHV-6 infection (high HHV-6 DNA load and plasma DNA–positive) failed to show a serologic response. This may be due, in part, to the suppressive effect of residual maternal antibody (below the level of detection in our fluorescent antibody assay) on the immune response, as has been reported for some vaccines [19]. The effect of passively transferred maternal antibody in suppressing serologic responses to primary HHV-6 infection has been alluded to before [4]. Two of the patients without a serologic response to HHV-6 had detectable HHV-7 antibody in the acute plasma specimen (in the absence of HHV-7 DNA), and these titers became undetectable in the convalescent specimen. It is possible that passively acquired cross-reactive HHV-7 antibody may dampen the immune response to HHV-6 without offering protection. Alternatively, the poor immune response may be due to the immaturity of the immune system of the young infant. It is possible that a serologic response may have been detected if blood specimens later in convalescence were tested and more sensitive methods were used. In our study, the 6-week-old child who seroconverted did so late and only to low titer, in marked contrast to older children, who developed brisk and high antibody responses. It is reported that the switchover from IgM to IgG antibody production is delayed in neonates [19]. We looked only for IgG antibody in our patients, and it may well be that these infants produced good IgM responses. It may also be interesting to dissect out the immune response to individual viral antigens by using Western blot antibody assays in these patients.

All of those patients with a serologic response to HHV-7 also had definite evidence of concurrent HHV-6 infection. The HHV-7 serologic responses in the other 11 patients are presumed to be cross-reactive antibody generated by HHV-6 infection.

A number of factors discussed above may potentially confound the use of the HHV-6 DNA IgG profile as a marker of primary infection. If the profile of HHV-6 DNA–positive/IgG antibody–negative is observed in an immunocompetent child of any age, the interpretation is that of primary HHV-6 infection. However, a profile of DNA positive/IgG positive may potentially be falsely interpreted as past infection, because of either passive maternal antibody in the young child, cross-reacting HHV-7 antibody, or the early development of a primary HHV-6 antibody response. In this study, the confounding effect of HHV-7 cross-reactivity on the HHV-6 DNA/IgG profile did not pose a problem in children >3 months old. This is probably because HHV-7 infection occurs later in life than HHV-6 infection. It would be predicted that the DNA/IgG profile may not be reliable for the diagnosis of HHV-7 infection, and this indeed is what we observed. It is the consensus of most investigators that specimens collected early in the course of HHV-6 infection are seronegative or have low levels of antibody [15]. We have attempted to minimize the effect of early low-level antibody responses by screening at a dilution of 1/50 and by detecting only IgG class antibody. The HHV-6 DNA/IgG profile is validated only within 5 days of disease onset.

In patients with undifferentiated febrile illness, primary HHV-6 infection was responsible for 50% of hospital admissions under the age of 1 years. This incidence is somewhat higher than that reported in other studies [4], and this may be partly due to the fact that the emergency department in our hospital is not staffed by pediatricians and may have a lower threshold for hospitalizing infants. However, it does emphasize the disease burden caused by this virus. Methods for rapid diagnosis could contribute significantly to clinical management, could help allay parent anxiety, and would probably be cost-effective.

In conclusion, we document that an HHV-6 DNA/IgG profile of positive viral DNA in whole blood associated with negative IgG is a sensitive and specific method for the diagnosis of primary HHV-6 infection in the acute (i.e., within 5 days of onset) blood specimen. However, a DNA/IgG profile of past infection in children under the age of 3 months must be treated with caution. The detection of plasma DNA is an acceptable, albeit less sensitive, approach to diagnosing primary HHV-6 infection. However, it may not differentiate between primary infection and virus reactivation in response to an unrelated infection. HHV-6 DNA load in whole blood differentiates between active and past HHV-6 infection, although again, this may not discriminate between primary infection and reactivation. The virus load assays as used in this study are not applicable in routine clinical practice in their present form, but we are presently developing assays that measure virus load in a format more usable in the routine clinical laboratory.

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